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## REVIEW & INTERPRETATION

### Adaptations of Endophyte-Infected Cool-Season Grasses to Environmental Stresses: Mechanisms of Drought and Mineral Stress Tolerance

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#### ABSTRACT

Cool-season grasses infected with *Neotyphodium* spp. endophytes have an extraordinary impact on the ecology and economy of pasture and turf. A range of adaptations of endophyte-infected grasses to biotic and abiotic stresses has been identified but mechanisms of these adaptations are not clearly understood. In this review, we present recent research progress on endophyte-related mechanisms affecting abiotic (drought, mineral) and selected aspects of biotic stress tolerance in cool-season grasses. Endophytes induce mechanisms of drought avoidance (morphological adaptations), drought tolerance (physiological and biochemical adaptations), and drought recovery in infected grasses. Mineral nutrition (nitrogen, phosphorus, calcium) affects production of ergot alkaloids, thus understanding mechanisms involved in mineral economy of endophyte-infected grasses will help in developing management practices to reduce forage toxicity to livestock. Previous research resolved the role of endophyte in nitrogen (N) economy of tall fescue. We identified two endophyte-related mechanisms in tall fescue operating in response to phosphorus (P) deficiency. The mechanisms are altered root morphology (reduced root diameters and longer root hairs) and chemical modification of the rhizosphere resulting from exudation of phenolic-like compounds. These mechanisms were shown to benefit endophyte-infected plants grown under P deficiency. We also report a mechanism of aluminum (Al) sequestration on root surfaces in endophyte-infected tall fescue, which appears to be related to exudation of phenolic-like compounds with Al-chelating activity. Understanding mechanisms of abiotic stress tolerance in endophyte-infected grasses is essential for continued improvement and persistence of grasses for a range of applications, e.g., forage for semi-arid areas or cover plants for soil renovation.

IN THEIR EVOLUTION, grasses have developed symbiotic associations with fungi including mycorrhizal fungi that grow in or on the roots (Smith and Read, 1997), and fungi that systemically infect grass shoots. Among the latter are grass endophytes, defined as fungi that live their entire life cycle within the aerial portion of

the host grass, forming nonpathogenic, systemic, and usually intercellular associations (Bacon and De Battista, 1991). The best known grass endophytes are classified in the tribe Balansieae of the family Clavicipitaceae (Ascomycetes) (Diehl, 1950). Currently, seven genera have been identified including *Atkinsonella*, *Balansia*, *Balansiopsis*, *Echinodopsis*, *Epichloë*, *Myriogenospora* (White, 1994), and *Parepichloë* (White and Reddy, 1998). Morgan-Jones and Gams (1982) erected the section *Albo-lanosa* to accommodate the anamorphic (imperfect) stage of *Epichloë* spp. in the genus *Acremonium* that caused symptomless infections of grasses in the subfamily Pooideae (Siegel, 1993). Glenn et al. (1996) reclassified the genus *Acremonium* sect. *Albo-lanosa* in a new genus *Neotyphodium* to separate clavicipitaceous *Acremonium* (e-endophytes) from *Acremonium* with other affinities (usually saprotrophs). The most widely known *Neotyphodium* endophytes are *N. coenophialum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin, *N. lolii* (Latch, Christensen, and Samuels) Glenn, Bacon and Hanlin, and *N. uncinatum* (Gams, Petrini and Schmidt) Glenn, Bacon and Hanlin that colonize tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), and meadow fescue (*F. pratensis* Huds.), respectively. Studies with grasses led to the discovery of two other groups of symptomless fungal endophytes. One group (p-endophytes) consists of closely related *Gliocladium*-like endophytes (An et al., 1993) in perennial ryegrass (Latch et al., 1984; Philipson, 1991) and *Phialophora*-like endophytes in meadow fescue, *F. gigantea* (L.) Vill., and Arizona fescue (*F. arizonica* Vasey) (Schmidt, 1991; An et al., 1993). The p-endophytes are ordered to Eurotiales (Ascomycetes) (Siegel et al., 1995) and are not related to e-endophytes (Leuchtmann, 1992). The p-endophytes differ from e-endophytes in patterns of mycelium growth and leaf blade colonization, colonization of roots, ability to sporulate on the host grass (thus being naturally infective), no

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**Abbreviations:** DM, dry matter; PWUE, instantaneous water use efficiency; TW/DW, turgid weight:dry weight ratio; WUE, water use efficiency.

alkaloid production (Siegel et al., 1995), and negligible effects on host tolerance to biotic and abiotic stresses (Schmidt, 1993; Malinowski et al., 1997a). Both groups of endophytes may occur symbiotically in the same host plant (Latch et al., 1984; Schmidt, 1991). The third group of grass endophytes (a-endophytes) has been proposed to accommodate endophytes found in annual ryegrass (*L. multiflorum* Lam.) and *F. paniculata* L. (Naffaa et al., 1998a). These endophytes belong to the section Simplex of the genus *Acremonium* and are represented by parasitic species of *Acremonium* similar to *A. chilense*, an endophyte of orchardgrass (*Dactylis glomerata* L.) (Morgan-Jones et al., 1990). Similar to p-endophytes, effects of a-endophytes on physiological processes and ecology of cool-season grasses are much less understood than effects of *Neotyphodium* spp. endophytes (Morgan-Jones et al., 1990; Schmidt, 1993; Malinowski et al., 1997a; Naffaa et al., 1998a), and will not be discussed in this review.

Interactions between *Neotyphodium* spp. endophytes and cool-season grasses have been referred to as defensive mutualism (Clay, 1988). In these associations, endophytes produce a range of alkaloids (Porter, 1994) or stimulate the host grass to synthesize alkaloids and other secondary metabolites (Bush et al., 1997; Ju et al., 1998) that protect the symbiotum from mammalian, insect, and nematode herbivores. Mutualistic symbioses, however, result in a net benefit to each participant. By comparing genetically identical endophyte-infected with noninfected plants, such benefits to the grass host may be shown. In contrast, *Neotyphodium* spp. endophytes cannot exist without their grass hosts (cultured mycelia excepted); therefore, they are completely dependent on the host grass for protection and supply for nutrients and water. Wilkinson and Schardl (1997) revised the concept of mutualistic symbioses between *Neotyphodium* spp. endophytes and their grass hosts and defined them as asymptomatic symbioses. In such associations, the loss of sexuality is a fundamental cost in the long term in contrast to associations which have both means of reproduction (called balanced symbioses). Thus, the most important benefit to *Neotyphodium* spp. endophytes appears to be dissemination through seeds of the host plant. This may, in part, explain an often reported increase in fitness of endophyte-infected grasses. Apparently, maximizing host growth by endophyte is in the ecological interest of both partners. Recently, virus-like particles have been identified in the endophyte *Epichloë festucae* Leuchtman, Schardl and Siegel isolated from asymptomatic *F. rubra* L. plants (Zabalgoitia et al., 1998), suggesting a third component (a mycovirus) of endophyte–grass associations. Although the presence of viral RNA did not induce any phenotypic effects in its fungal host, possible phenotypic effects on the grass host deserve attention in future experiments.

Endophyte-infected grasses express a range of adaptations to biotic (reviewed in Latch, 1993) and abiotic stresses, including drought (reviewed in Bacon, 1993; West, 1994), mineral imbalance (Lyons et al., 1990; Malinowski et al., 1998a, 1999b), and soil acidity (Belesky and Fedders, 1995; Malinowski and Belesky, 1999a). As a result, endophyte-infected grasses are more competi-

tive than noninfected grasses and thrive better than noninfected grasses with limited resources (Marks et al., 1991; Bacon and Hill, 1996; Hill et al., 1998). Recent approaches to endophyte–grass interactions focus on isolated endophyte strains that, in association with grasses, show minimal or no production of alkaloids toxic to livestock yet retain the pest- and drought-resistance benefits of symbiotic plants (Fletcher and Easton, 1997; Funk and White, 1997; Bacon and Hinton, 1998). These associations are crucial for improved livestock performance on fescue and ryegrass. Once alkaloid production has been altered, it is essential to understand mechanisms involved in abiotic stress tolerance of endophyte-infected grasses for their continued improvement and persistence for a range of applications.

We outline the principal research findings and conclusions on abiotic and biotic stress tolerance of *Neotyphodium* spp. endophyte–grass associations, emphasizing recent research progress on endophyte-induced mechanisms of mineral stress tolerance in tall fescue.

### Mechanisms of Biotic Stress Tolerance

Persistence of individual plants and ultimately populations in grassland and pasture ecosystems is determined by biotic and abiotic factors (reviewed in Briske and Heitschmidt, 1991; Briske, 1996) interacting with population genetics. Biotic factors include herbivores (animals, insects), nematodes, and pathogens (fungi, bacteria, and viruses). Herbivory can influence the canopy environment by altering light conditions, nutrient cycling dynamics, and water use. Because endophyte-infected and noninfected grasses respond differently to such changes in canopy environment, herbivory, therefore, can interact with endophyte infection to determine productivity of grasses. There is a strong selective advantage for endophyte-infected grasses in populations that include noninfected plants (Clay, 1988). This is because endophyte infection stimulates production of an array of bioprotective alkaloids (Porter, 1994; Bush et al., 1997) and non-alkaloid secondary metabolites (Koshino et al., 1989; Ju et al., 1998) in the host grass. These metabolites can deter livestock and insect herbivores (Breen, 1994; Ball, 1997). A relationship between health disorders in cattle and a high level of endophyte infestation in tall fescue from toxic pastures in the USA was first shown by Bacon et al. (1977). A similar relationship was later reported for perennial ryegrass in New Zealand (Fletcher and Harvey, 1981) and other grass species infected with *Neotyphodium* spp. endophytes (Petroski et al., 1992; Miles et al., 1998).

Researchers identified several alkaloids responsible for poor performance of animals grazed or fed on endophyte-infected grasses (Porter, 1994). Ergot alkaloids (clavines, lysergic acid and its amides, ergopeptines) are responsible for fescue toxicosis in grazing livestock and are produced by endophytes of tall fescue and perennial ryegrass, *N. coenophialum* and *N. lolii*, respectively. When artificially infected with *N. coenophialum*, meadow fescue can also synthesize ergot alkaloids in contrast to the natural *N. uncinatum*-meadow fescue association, which does not (Christensen, 1997). Ergot

alkaloids were detected in many endophyte-infected grass species other than *Lolium* spp. and *Festuca* spp. (Bacon et al., 1986; Miles et al., 1996). Lolitrem (indole diterpene) alkaloids [paxilline, paxitriol(s), lolitriol, lolitrems] were first identified in symbiotic ryegrass plants and related to livestock neurotoxicities known as *ryegrass staggers*. The ability to produce lolitrems and paxilline has also been shown in vitro for cultures of *N. coenophialum* and *N. uncinatum* (Penn et al., 1993), and in vivo in endophyte-infected tall fescue (Garthwaite, 1997). The pyrrolopyrazine alkaloid peramine is produced by endophyte-infected ryegrass, tall fescue, and other grasses (Siegel et al., 1990). Peramine has insect-feeding deterrent properties (Breen, 1994), but no apparent activity against mammalian herbivores (Bush et al., 1997). Loline alkaloids (*N*-acetyllooline, *N*-formyllooline) are synthesized by endophyte-infected grasses (Siegel et al., 1990; Porter, 1994) and they are potent insecticides (Dahlman et al., 1997). In endophyte-infected meadow fescue and tall fescue, the fungal component of the association seems to play a key role in synthesis of loline alkaloids (Wilkinson et al., 1997). Huizing et al. (1991) documented involvement of high temperatures in loline alkaloid production by endophyte-infected perennial ryegrass. Loline alkaloids have negligible effects on livestock compared with ergot or lolitrem alkaloids (Strickland et al., 1996), although their concentrations in endophyte-infected grasses are 100 to 1000 times higher than those of other alkaloids (Siegel et al., 1989). Indole alkaloids (indoleglycerol, indoleacetic acid) are products of tryptophan metabolism of endophyte, plant, and the symbiotum. Their effects on animal performance are not known. Indole alkaloids have the potential to interfere with host plant physiology and synthesis of other secondary metabolites by the symbiotum. Other miscellaneous alkaloids (ergosterol, ergostetraeneone, ergosterol-peroxide, harman, norharman, halostachine) found in endophyte-infected grasses may be involved in animal toxicities and may act as phytoalexins (antibiotics) as well (Latch, 1993).

Non-alkaloid secondary metabolites occurring in timothy (*Phleum pratense* L.) infected with *E. typhina* (Pers. : Fr.) have been classified as sesquiterpenoids, fatty acids, and phenolic compounds (Yoshihara et al., 1985; Koshino et al., 1988, 1989), while flavonoids have been detected in bluegrass (*Poa ampla* Merr.) infected with *N. typhinum* (Morgan-Jones and Gams) Glenn, Bacon and Hanlin (Ju et al., 1998). The ecological significance of these compounds in terms of biotic stress tolerance of endophyte-infected grasses is unknown.

Endophytes can indirectly reduce insect-transmitted viral diseases in grasses (West et al., 1990). Nonendophyte-infected grasses are more resistant than noninfected grasses to soil-borne nematodes (West et al., 1988; Eerens et al., 1997), with resistance probably attributable to alkaloids present in roots (Malinowski et al., 1998b, 1999a). Increased secretion of phenolic-like compounds from roots of infected tall fescue (Malinowski et al., 1998a) could act as feeding deterrents in the rhizosphere, although this remains to be resolved. The reader

is referred to Bacon et al. (1997) for a thorough review of endophyte-grass association effects on biotic factors including bacteria, fungi, nematodes, insects, and non-domesticated mammals.

Endophyte-infected grasses, in general, compete successfully with non-infected conspecifics as well as with other plant species and, in time, can dominate a sward (Clay and Holah, 1999). In some cases, the competitive advantage may result from morphological responses such as more massive and more numerous tillers (Hill et al., 1990), greater leaf elongation rate (Eerens et al., 1993; Malinowski et al., 1997b), and altered root architecture (Malinowski et al., 1999b). In others, endophyte-infected grasses may have direct chemical effects (allelopathy) on the competitor. For example, the number of white clover (*Trifolium repens* L.) plants declined in pastures dominated by endophyte-infected, compared with non-infected, perennial ryegrass (Percival and Duder, 1983; Sutherland and Hoglund, 1989). Seed extracts of endophyte-infected tall fescue inhibited germination of *Trifolium* spp. (Springer, 1997). Strong suppression of leguminous companion species by endophyte-infected tall fescue in a mixed sward could have significant negative effects on productivity and N economy of a sward. Loline alkaloids may be one such group of endophyte-related secondary metabolites with allelopathic properties. Despite their role as insect deterrents, loline alkaloids were the only group of endophyte-related alkaloids shown to reduce germination rate of monocot and dicot seeds (Petroski et al., 1990); therefore, loline alkaloids may enhance the competitive ability of endophyte-infected grasses by retarding the establishment of competitors in a sward. Recently, allelopathic effects of lolines in endophyte-infected tall fescue-competitor associations have been reported by Bush et al. (1997) and Malinowski et al. (1999a). Malinowski et al. (1999a) observed that loline alkaloid concentration in roots of endophyte-infected tall fescue genotype DN2 was twice that detected in genotype DN11 (1083  $\mu\text{g g}^{-1}$  and 536  $\mu\text{g g}^{-1}$ , respectively). When both tall fescue genotypes were grown in binary mixtures with red clover (*Trifolium pratense* L.), the relative yield total (RYT) of the red clover/DN2 tall fescue mixture was less than 1.0 in response to endophyte infection (Fig. 1). This suggested an allelopathic interaction between these two components of the mixture (Trenbath, 1974). In contrast, the RYT values of red clover binary mixtures with endophyte-infected DN11 and noninfected DN2 and DN11 tall fescue were not significantly different from 1.0, indicating full competition for the same limiting resources (Snaydon and Sattore, 1989). Detailed research with additional tall fescue genotypes is needed to resolve the role of loline alkaloids in allelopathic effects of endophyte-infected grasses on other plant species.

Current efforts focus on development of endophytes which, in association with grass hosts, produce little or no ergot alkaloids toxic to livestock, yet retain pest- and drought-resistance attributes of symbiotic plants. The impact of endophyte-mediated surrogate transformation (Bacon and Hinton, 1998) on mechanisms of adap-



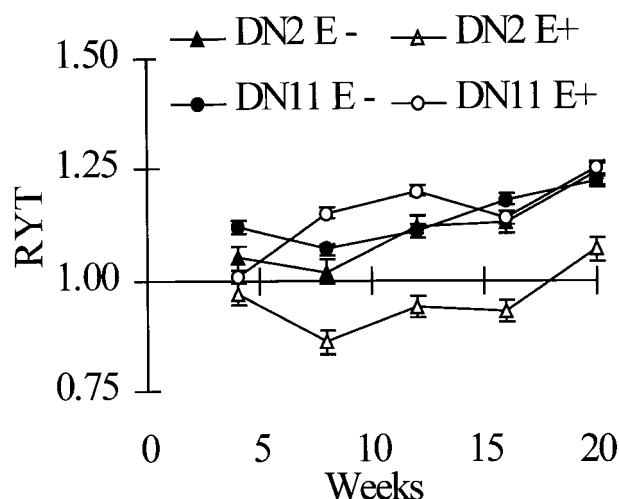


Fig. 1. Relative yield total (RYT) of red clover and endophyte-infected (E+) and noninfected (E-) tall fescue genotypes DN2 and DN11 grown in binary mixtures. When endophyte-infected, the concentration of loline alkaloids in roots of DN2 was twice that of DN11, indicating a possible allelopathic effects on red clover. Adapted from Malinowski et al. (1999a).

tation and host survival in competitive environment and marginal edaphic conditions is unknown and warrants investigation.

### Adaptations and Mechanisms of Drought Stress Tolerance

Drought stress tolerance is the most thoroughly documented attribute of abiotic stress tolerance in endophyte-infected grasses. Drought stress in temperate environments is usually a result of water deficit in the soil and high temperature. Water deficit induces a range of adaptations in plants that favor their growth or survival (Arrau deau, 1989). These adaptations include mechanisms of drought avoidance, tolerance, and recovery from drought. In our discussion, we will show that infection with *Neotyphodium* spp. endophytes may induce some adaptations and mechanisms of drought stress tolerance in cool-season grasses and ensure a better survival than noninfected conspecifics.

#### Drought Avoidance

Drought-avoiding plants possess a variety of morphological and physiological adaptations to maintain favorable water status, either by maintaining an efficient water supply to aboveground organs or conserving water during periods of soil water deficit (Clarke and Durley, 1981). The adaptations may be divided into three basic groups: (i) improved water uptake from the soil by an extensive root system, (ii) reduced transpiration losses, and (iii) water storage in plant tissues.

**Extensive Root System.** Improved water uptake from the soil may be promoted by a deeper and denser root system (Arrau deau, 1989). In response to endophyte infection, increased root dry matter (DM) has frequently been reported in perennial ryegrass (Latch et al., 1985), tall fescue (De Battista et al., 1990), and meadow fescue (Malinowski et al., 1997a, b). A direct relationship between the size of root system and drought tolerance, however, has not been documented for endo-

phyte-infected grasses. Responses of grass root system to infection with shoot-located endophytes are not easy to quantify because of difficulties in measuring root characteristics. Malinowski et al. (1999b) showed that endophyte infection increased root hair length and decreased root diameter in tall fescue. These traits could increase root surface area for water and mineral acquisition. Because roots are the first plant organs to sense water depletion in the soil, further research on root activity of endophyte-infected grasses should clarify the hypothesis of a water stress signaling system existing in endophyte-infected grasses (West et al., 1994).

**Control of Transpiration.** Water deficits in plants induce stomatal closure, which may benefit plants in the short term by reducing water loss via transpiration (Turner, 1986). Endophyte presence in shoots was shown to affect stomatal behavior in tall fescue (Elmi and West, 1995; Buck et al., 1997) and meadow fescue (Malinowski et al., 1997a, b), but apparently not in perennial ryegrass (Barker et al., 1997). Stomatal conductance of water-stressed endophyte-infected fescues (*Festuca* spp.) declined earlier and faster than that of noninfected plants, suggesting more rapid stomatal closing (Elbersen et al., 1994; Elmi and West, 1995). The direct mechanism of this phenomenon is not known. A biochemical signal from the endophyte or an altered hormonal status in the host may predispose leaf blades to respond quickly to early stages of water deficit (Belesky et al., 1987a; West et al., 1994). The hypothesized biochemical signal induced in endophyte-grass associations in response to water deficit may be interpreted as an endophyte-related perturbation in host grass homeostasis. Endophyte, as a xenobiotic component of a grass plant, may induce a permanent internal stress in the host grass. This may precondition or sensitize the plant to drought and other stresses, thereby permitting the plant to exhibit adaptive responses (such as stomata closure) sooner than noninfected conspecifics. Recent research results on symptomless fungal endophytes and their host plants suggest mutual defense responses involving a range of secondary metabolites produced in much higher quantities than observed in plant-fungal pathogen interactions (Schulz et al., 1999). The authors hypothesize that both the pathogen-host and the endophyte-host interactions involve constant mutual antagonisms at least in part based on the secondary metabolites the partners produce. Whereas the pathogen-host interaction is imbalanced and results in a disease, that of the endophyte and its host is a balanced antagonism. Responses suggest that host grasses recognize *Neotyphodium* spp. endophyte infection by inducing a range of defense mechanisms similar to those against pathogenic fungi. Circumstantial evidence for defense mechanisms of grasses to endophyte infection includes the presence of chitinase (a pathogenesis-related protein) (Roberts et al., 1992), resveratrol (a phenolic compound with antifungal activity correlated with disease resistance) (Powell et al., 1994), and increased concentration of phenolic-like compounds in shoots and roots compared with noninfected grasses (Malinowski et al., 1998a). Novel endophyte-grass associations may be highly incompatible (Koga et al., 1993; Christensen et al., 1995). This incompatibility may be a result of fungal

problems with enzymatic degradation of the middle lamella of host cell walls, which in turn would restrict the nutritional supply within the apoplast of host tissues (Leuchtmann, 1994), also suggesting multifaceted defense responses.

**Water Storage in Plant Tissues.** Water content of tiller bases of some endophyte-grass associations may be maintained at higher levels than those in noninfected plants during drought (Elbersen and West, 1996; Buck et al., 1997). This phenomenon may be explained by enhanced accumulation of solutes in tissues of endophyte-infected plants compared with noninfected plants, or by reduced leaf conductance and a slow-down of the transpiration stream, or thicker cuticle.

### Drought Tolerance

Drought tolerance refers to physiological and biochemical adaptations that enable plant tissues to withstand water deficits (Clarke and Durley, 1981). In most grasses, a combination of drought avoidance and tolerance mechanisms determines the survival potential during drought. Mechanisms of drought tolerance have been divided into three major groups: (i) accumulation and translocation of assimilates, (ii) osmotic adjustment, and (iii) maintenance of cell wall elasticity (Arrau deau, 1989). All these mechanisms can be affected by endophyte infection in grasses.

**Accumulation and Translocation of Assimilates.** Accumulation of carbohydrates during growth under non-drought conditions and translocation during imposed drought stress may contribute to drought tolerance. In some endophyte-tall fescue associations, endophyte-infected plants had greater concentrations of total non-structural carbohydrates in tillers than noninfected plants when grown under non-limiting water supply (Hill et al., 1990). Water-stressed endophyte-infected plants of tall fescue genotype CB1 accumulated more glucose and fructose in leaf blades and leaf sheaths than noninfected plants (Richardson et al., 1992). These carbohydrates are osmotically active and may play a role in endophyte-related osmotic adjustment in tall fescue.

**Osmotic Adjustment.** Accumulation of solutes in tissues in response to drought helps maintain turgor and facilitates physiological and biochemical processes. The following groups of solutes have been particularly considered to contribute to osmotic adjustment in endophyte-infected grasses.

1. Water-soluble sugars (Richardson et al., 1992).
2. Fungal metabolites mannitol and arabitol (polyols) (Richardson et al., 1992).
3. Amino acid proline. Accumulation of proline in plant tissues in response to drought is common and is correlated with drought resistance in many crops (Hsiao, 1973). Proline may be an osmotically active metabolite (Joyce et al., 1992). Increased proline concentration was not related to osmotic adjustment in drought stressed *Lolium* spp. and *Festuca* spp. grasses (Thomas, 1991; Barker et al., 1993; Abernethy and McManus, 1998; Abernethy et al., 1998). Belesky et al. (1984) likewise did not find a drought-stress related proline accumulation in tall fescue of unspecified endophyte infection

status. In contrast, Elbersen and West (1996) observed lower proline concentrations in three drought-stressed endophyte-infected genotypes of tall fescue compared with their noninfected isolines in a field experiment, suggesting that endophyte-infected plants were less stressed than non-infected plants. Under field conditions, however, lower concentrations of proline in endophyte-infected tall fescue plants could be related not only to water stress but to other factors (e.g., mineral status) as well. In meadow fescue, proline accumulation during imposed single water stress cycle was not affected by endophyte, but proline metabolism (proline degradation rate) during recovery was slower in endophyte-infected plants compared with genetically identical, noninfected plants (Malinowski, 1995). This might suggest a residual effect which predisposes endophyte-infected plants to rapid adjustment when undergoing subsequent drought stress events. Proline metabolism may be affected in endophyte-infected grasses because proline is a product of ergot alkaloid breakdown (Stoll and Hofmann, 1965) and peramine synthesis (Siegel et al., 1990).

4. Other amino acids. Belesky et al. (1984) showed that water stress increased accumulation of amino acids in endophyte-infected tall fescue but the results were not compared to noninfected plants. Nitrogen fertilization increased amino acid concentrations in endophyte-infected, compared to noninfected, tall fescue (Lyons et al., 1990), with greater accumulation in leaf sheaths (where the fungal hyphae are present in great number) than blades (less frequent hyphae).
5. Loline alkaloids. Loline alkaloids are produced by some endophyte-infected grass species (Bush et al., 1993). Because loline alkaloids are water soluble, they may act as osmoregulators (Bacon, 1993). The concentration of loline alkaloids in plant tissues may exceed 8 g kg<sup>-1</sup> DM, which could affect osmotic potential (Kennedy and Bush, 1983; Bush et al., 1993). The presence of loline alkaloids in roots of endophyte-infected tall fescue (Bush et al., 1993; Malinowski et al., 1999a) suggests that, once metabolized by the shoot-located fungus, they may be translocated within the plant to zones lacking the fungus, such as roots and meristematic tissues (Justus et al., 1997). The evidence for involvement of loline alkaloids in drought tolerance of endophyte-infected grasses is circumstantial at this time (Bacon and Hinton, 1998). Endophyte-infected tall fescue and meadow fescue contain high levels of loline alkaloids, and both grasses express greater drought tolerance than do noninfected plants (Bacon, 1993; Malinowski et al., 1997a,b). In contrast, endophyte-related increase in drought tolerance could not be confirmed for perennial ryegrass (Barker et al., 1997), where loline alkaloids occur in low concentrations. The concentration of loline alkaloids in tall fescue increased in response to soil water deficit (Belesky et al., 1989b) and high temperature during summer

(Robbins et al., 1973). In contrast, Kennedy and Bush (1983) could not confirm high temperature effects on loline alkaloid accumulation in tall fescue in growth chamber experiments. The influence of high temperature on loline alkaloid synthesis in perennial ryegrass was shown by Huizing et al. (1991) and may explain the absence of loline alkaloids in perennial ryegrass in previous studies conducted under optimal temperatures for cool-season perennial grasses (Gleason et al., 1990). Production of loline alkaloids in field-grown tall fescue and meadow fescue varied seasonally (Belesky et al., 1987b; Justus et al., 1997). In endophyte-infected tall fescue, loline alkaloids are accumulated in greater amounts in response to drought and during recovery from drought (Bush et al., 1993, 1997).

Future research should resolve the role of loline alkaloids in drought tolerance of cool-season grasses. One approach may be a comparison of drought stress responses between endophyte-infected plants producing loline alkaloids and endophyte-infected plants with a disturbed pattern of loline alkaloid synthesis. Loline alkaloids are synthesized from two polyamines, putrescine and spermidine (Bush et al., 1993; Graser and Hartmann, 1997). Spermidine is supposed to be the precursor of the pyrrolizidine ring system of loline alkaloids (Bush et al., 1993). At this time, it is not known if the loline alkaloid precursors putrescine and spermidine are of fungal or host origin. If loline alkaloids were produced from fungal-derived polyamines, a disturbed pattern of polyamine synthesis by endophyte might affect synthesis of loline alkaloids in the symbiotum. This could create a basis for a precise identification of physiological mechanisms of abiotic stress tolerance in endophyte-infected grasses directly related to loline alkaloid action. Certain chemicals have been shown to disturb polyamine synthesis in fungi with negligible effects on plant-synthesized polyamines and plant growth (Elghachtouli et al., 1996; Walters and Macintosh, 1997). For example,  $\alpha$ -difluoromethylornithine (DFMO) is an inhibitor of ornithine decarboxylase, and  $\alpha$ -difluoromethylarginine (DFMA) inhibits arginine decarboxylase (Walters and Macintosh, 1997). These inhibitors may suppress polyamine synthesis by endophyte and affect loline alkaloid production in the host. If endophyte could synthesize loline alkaloids from grass-originated polyamines, fungal polyamine inhibitors might not affect loline alkaloid synthesis in infected grasses. To date, there is no published information of endophyte effects on polyamine accumulation and involvement of polyamines in drought stress-related accumulation of loline alkaloids in infected grasses.

**Maintaining Cell Wall Elasticity.** Cellular (tissue) elasticity enables cell size to change in synchrony with water availability (Arrau deau, 1989). White et al. (1992) showed that endophyte infection lowered bulk modulus of tissue elasticity (suggesting less rigid cell walls) and increased turgid weight:dry weight (TW/DW) ratio in water stressed tall fescue. The TW/DW determines water uptake capacity and reflects the ability of cells to

absorb water. Higher values of the TW/DW ratio indicate less damaged cell walls. Similar to tall fescue, the TW/DW ratio in meadow fescue leaves was also higher in response to endophyte infection during imposed water stress (Malinowski, 1995). These results suggest that in response to water stress, endophyte may alter cell wall characteristics in a similar way in both grass species. At this time, it is not known which metabolites are directly involved in the process.

### Water Use Efficiency

One adaptation to drought stress in plants is efficient water use. Considerable research has been conducted concerning limitations to efficient water use in grass production and improving water use efficiency (WUE) in grasses (reviewed in Johnson and Asay, 1993). The WUE measures the ability to limit water loss while maintaining net carbon uptake in the leaves. Because endophyte-infected grasses can reduce water loss through stomatal closure during soil drying, they may be better able to prolong assimilation or resume growth after drought more rapidly than noninfected grasses (West, 1994). Experimental results are not consistent in support of this hypothesis (Belesky et al., 1987a; Marks and Clay, 1996). Under water non-limiting conditions in a greenhouse, instantaneous water use efficiency (PWUE, ratio of photosynthesis:transpiration rates on unit leaf area basis) calculated from data reported by Richardson et al. (1990) tended to be higher in leaves of endophyte-infected tall fescue genotypes than in non-infected conspecifics. In contrast, PWUE values of drought-stressed endophyte-infected tall fescue genotypes were lower than those of noninfected plants (Bates and Joost, 1990). The latter results showed, however, that noninfected tall fescue plants reduced PWUE more rapidly than endophyte-infected plants as soil water deficit increased, suggesting less efficient adaptation to water stress.

In cool-season grasses and other plant species that fix carbon by the  $C_3$  pathway, WUE is related to the stable  $^{13}C$  isotope composition of tissues and can be indirectly estimated from the  $^{13}C$  to  $^{12}C$  ratio in the biomass (Johnson and Bassett, 1991). Plants discriminate against  $^{13}C$  during the process of photosynthesis and the  $^{13}C$  discrimination ( $\Delta$ ) is described as:

$$\Delta = \left( \frac{^{13}CO_2/^{12}CO_2 [\text{sample}]}{^{13}CO_2/^{12}CO_2 [\text{standard}]} - 1 \right) \times 1000 \quad (\text{Tieszen and Grant, 1990}).$$

Theoretically, the  $\Delta$  gives an integrated estimate of the ratio of leaf intercellular  $CO_2$  concentration ( $C_i$ ) to ambient  $CO_2$  concentration ( $C_a$ ) at constant vapor pressure difference (Johnson and Bassett, 1991). Drought-stressed endophyte-infected grasses have a reduced transpiration rate because of a more rapid stomatal closure than noninfected plants (Elbersen et al., 1994; Elmi and West, 1995). Therefore, one would also expect a reduced carbon fixation rate in endophyte-infected grasses due to a lower  $C_i/C_a$  ratio and, in consequence, higher  $\Delta$  values (Johnson, 1993). Because the  $\Delta$  is negatively correlated with WUE (Farquhar et al., 1989; Read et al., 1991), endophyte-infected grasses are expected



to have lower WUE than noninfected plants under drought. A limited number of experiments on  $\Delta$  and WUE in endophyte-infected grasses do confirm this hypothesis. In a population of Kentucky-31 tall fescue, Johnson and Tieszen (1993) observed lower mean  $\Delta$  in noninfected plants compared with endophyte-infected plants in response to drought. Although the authors considered this result as preliminary, it might suggest that endophyte-infected plants had lower WUE than noninfected plants. In other words, endophyte-infected plants might be more conservative than noninfected plants by reducing growth during drought and conserving water in the soil-plant system. Detailed results on WUE in perennial ryegrass confirmed that endophyte-infected plants had lower WUE than noninfected plants in response to drought (Eerens et al., 1998). Interestingly, endophyte-infected plants of perennial ryegrass were less wilted (thus less stressed) than noninfected plants because of improved water status characteristics. Results suggest that endophytes do not directly affect water use efficiency in drought-stressed grasses. Endophytes appear to help maintain physiological and biochemical processes at the cost of reduced growth during drought and facilitate rapid regrowth of host grasses soon after soil water becomes available.

Recovery mechanisms determine the ability of plants to regrow when drought stress is relieved. In endophyte-infected grasses, these mechanisms include a rapid water uptake by roots and the ability of tissues to restore physiological functions. Endophyte effects on growth and water relations in grasses were observed during the weeks following water stress (West et al., 1994; Malinowski et al., 1997b). West et al. (1994) postulated that endophyte effects on growth and drought tolerance may be more important for grass persistence during recovery from drought than during the onset of drought.

Although responses of endophyte-infected grasses to drought have been well documented, the understanding of mechanisms by which endophytes enhance host grass survival during drought is incomplete. Endophyte infection of grasses induces a range of responses to water deficit that are a combination of drought avoidance, tolerance and recovery mechanisms. These responses may or may not be expressed by a particular endophyte-grass association, but the sum of responses of individual endophyte-grass associations determines the ecological success of a given grass population (West, 1994). As a result, the range of adaptation of endophyte-infected cool-season grasses can be extended into areas dominated by warm-season grasses which can help improve pasture productivity (Bouton et al., 1993; Cunningham et al., 1994).

### **Adaptations and Mechanisms of Mineral Stress Tolerance**

Alkaloids in tall fescue were reported long before the discovery of its associated endophyte (Yates, 1962, 1963), and before close relationships between alkaloid concentrations in tall fescue herbage and mineral nutrition were identified. For example, greater N fertilization increased concentrations of perloine while P and K

fertilization reduced concentration of this alkaloid (Gentry et al., 1969). After the discovery of endophyte and its role in alkaloid synthesis in the host grass, management practices were developed to reduce alkaloid production and toxicity of tall fescue. One of the first management strategies was manipulating N nutrition.

### **Nitrogen**

High rates of N fertilizer increased the concentration of ergopeptine alkaloids in controlled-environment (Lyons and Bacon, 1984) and field grown endophyte-infected tall fescue (Belesky et al., 1988). Increasing N fertilization from 134 kg ha<sup>-1</sup> to 334 kg ha<sup>-1</sup> increased the concentration of total ergopeptine alkaloids by 60% to 80%, depending on the year (Belesky et al., 1988). Using a single clone of Kentucky-31 tall fescue, Arechavaleta et al. (1992) showed that accumulation of ergot alkaloids increased not only in response to N fertilization but was also dependent on N source. In that experiment, ergot alkaloids were not detected at a very low N level in the soil (11 mg pot<sup>-1</sup>). In contrast, concentrations of ergovaline and total ergopeptides were 0.41 and 0.57  $\mu\text{g mg}^{-1}$  DM, respectively, at a high N level (220 mg pot<sup>-1</sup>). The NH<sub>4</sub><sup>+</sup> form of applied N had a greater effect on accumulation of ergot alkaloids than the NO<sub>3</sub><sup>-</sup> form. The concentration of loline alkaloids is also affected by N fertilization. Belesky et al. (1987b) showed that greater loline alkaloid concentrations on a unit of endophyte infection rate were associated with increased N availability in the soil.

It is well known that N fertilization increases herbage yield of grasses. Clay (1987) analyzed endophyte effects on DM production of tall fescue and perennial ryegrass seedlings grown under optimal nutrient supply (weekly applied soluble fertilizer 20:20:20 NPK, 1 g L<sup>-1</sup>). For both species, tiller number and above-ground biomass of endophyte-infected plants were greater than those of noninfected plants. Cheplick et al. (1989) found that DM production by seedlings of tall fescue and perennial ryegrass depended on nutrient availability and endophyte infection. Seedling DM of endophyte-infected ryegrass was greater at intermediate (biweekly applied soluble fertilizer 20:20:20 NPK, 0.15 g L<sup>-1</sup>) and high (1.5 g L<sup>-1</sup>) nutrient levels compared with noninfected plants but no differences in plant biomass were observed when no nutrients were added. In contrast, endophyte infection reduced biomass of tall fescue seedlings at low nutrient level but increased plant biomass at high nutrient level. Endophyte-infected adult plants of tall fescue produced more biomass than did noninfected plants, whereas adult ryegrass plants did not respond to endophyte infection, regardless of nutrient availability. On the basis of these results, the authors concluded that endophyte infection might induce an additional metabolic cost to the host plant grown under mineral stress conditions. Plants used by Cheplick et al. (1989) represented a population. As such, they varied in the genetic make up, thus endophyte-related effects on growth were inconsistent. Belesky et al. (1991) showed that endophyte did not affect carbohydrate accumulation in field-grown tall fescue populations grown either at low (134

kg ha<sup>-1</sup>) or high (336 kg ha<sup>-1</sup>) N availability. The authors concluded that endophyte was not detrimental to the host grass grown at low soil N level. Working on genetically identical plants, Belesky et al. (1989a) found that four of five tall fescue clones produced more biomass (15% on the average) in response to endophyte infection when grown at optimal N level of 150 mg kg<sup>-1</sup> soil and adequate water supply. A detailed study by Arachevaleta et al. (1989) showed that N-use efficiency was greater in endophyte-infected plants. In response to endophyte infection, plants of a tall fescue clone derived from cv. Kentucky-31 grown at low (11 mg pot<sup>-1</sup>) N level produced a similar herbage DM to noninfected plants grown at high (220 mg pot<sup>-1</sup>) N level. With increased N supply, the difference between endophyte-infected and noninfected plants became more dramatic as shown by a 100% and a 50% increase in herbage DM, respectively for plants grown at 73 and 220 mg N pot<sup>-1</sup>.

Proline and amino acid concentrations in Kentucky-31 tall fescue populations (the degree of endophyte infection not determined) depended on soil N availability (Belesky et al., 1982, 1984). Similarly, amino acid concentrations were greater in endophyte-infected than in noninfected tall fescue as N fertilization increased (Belesky et al., 1984). Lyons et al. (1990) observed a greater accumulation of amino acids in leaf sheaths than blades of tall fescue. An endophyte-related increase in total amino acid concentration was significant only at high N level (10 mM) but no effects were observed at low N level (0.5 mM). They found an endophyte-related increase in sheath NH<sub>4</sub><sup>+</sup> concentrations and decrease in sheath and blade NO<sub>3</sub><sup>-</sup> concentrations, which suggested an endophyte effect on nitrogen metabolism in tall fescue. A detailed investigation showed that endophyte-infected plants had greater activity of glutamine synthetase, an enzyme responsible for NH<sub>4</sub><sup>+</sup> reassimilation, regardless of N availability. This mechanism helped explain a more efficient use of N by endophyte-infected tall fescue as previously shown by Arachevaleta et al. (1989). Alteration of N metabolism by endophyte in tall fescue appears to involve both assimilatory and basic nitrogen metabolism (Lyons et al., 1990) and may be correlated with mechanisms of *in vitro* N utilization by endophyte mycelium. Kulkarni and Nielsen (1986) showed that a *N. coenophialum* strain could assimilate NH<sub>4</sub><sup>+</sup> but not NO<sub>3</sub><sup>-</sup> as a source of N from the growth medium. In contrast, three out of four *N. coenophialum* isolates from Kentucky-31 tall fescue analyzed by Ferguson et al. (1993) could utilize NO<sub>3</sub><sup>-</sup> and all of them could utilize NH<sub>4</sub><sup>+</sup> as N source. Naffaa et al. (1998b) examined growth of endophytes from 12 grass species of Poaceae and found that all of them (including *N. lolii*) could assimilate NH<sub>4</sub><sup>+</sup> but only *A. chilense*-like endophytes (a-endophytes) could assimilate NO<sub>3</sub><sup>-</sup>. These results suggest a considerable variability in N utilization among endophyte isolates from different grass species. It remains to be resolved if increased N use efficiency in grasses could be directly (fungal enzymes) or indirectly (stimulation of the host enzymes) related to endophyte infection.

Responses of endophyte-infected grasses, other than tall fescue, to N nutrition are less well studied. In a

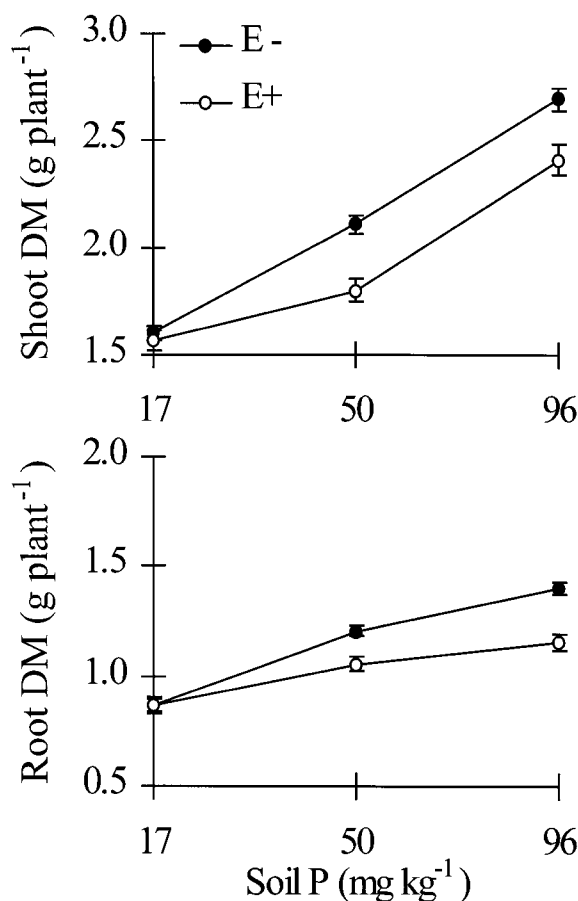


Fig. 2. Root and shoot DM of endophyte-infected (E+) and noninfected (E-) tall fescue (averaged over four genotypes) as a function of soil P availability. Adapted from Malinowski et al. (1998b).

single clone of perennial ryegrass grown in nutrient solutions at low (3  $\mu$ M NH<sub>4</sub>NO<sub>3</sub>) and high (30  $\mu$ M NH<sub>4</sub>NO<sub>3</sub>) N levels, endophyte infection did not affect either DM production or N uptake (Lewis et al., 1996). In an experiment by Ravel et al. (1997) with three clones of perennial ryegrass, endophyte-infected plants had more leaves and tended to have more tillers than noninfected plants when grown at low (2.33 mM) N level in the soil. Dry matter yield, however, was not affected by endophyte, confirming results by Lewis et al. (1996). They suggested that photosynthesis was similar for endophyte-infected and noninfected plants grown under N deficiency. Interestingly, endophyte-infected plants compensated for lower net photosynthesis with an increased number of green leaves compared with noninfected plants. Malinowski (1995) observed no significant effect of endophyte on N use efficiency (DM produced per unit of uptaken N) of meadow fescue grown under nonstressed (daily supply with 7.5 mM N nutrient solution) conditions, regardless of soil water content. Cloned genotypes of Arizona fescue infected with a *Neotyphodium* spp. endophyte had a greater herbage DM at low soil N level but no responses were observed at high soil N level compared with noninfected plants (Louis and Faeth, 1997). In a subsequent study with grazed populations of Arizona fescue, Schulthess and Faeth (1998) found more *N. starri*-infected plants at a location with the lowest soil N concentration (about 0.6 mg kg<sup>-1</sup>) than



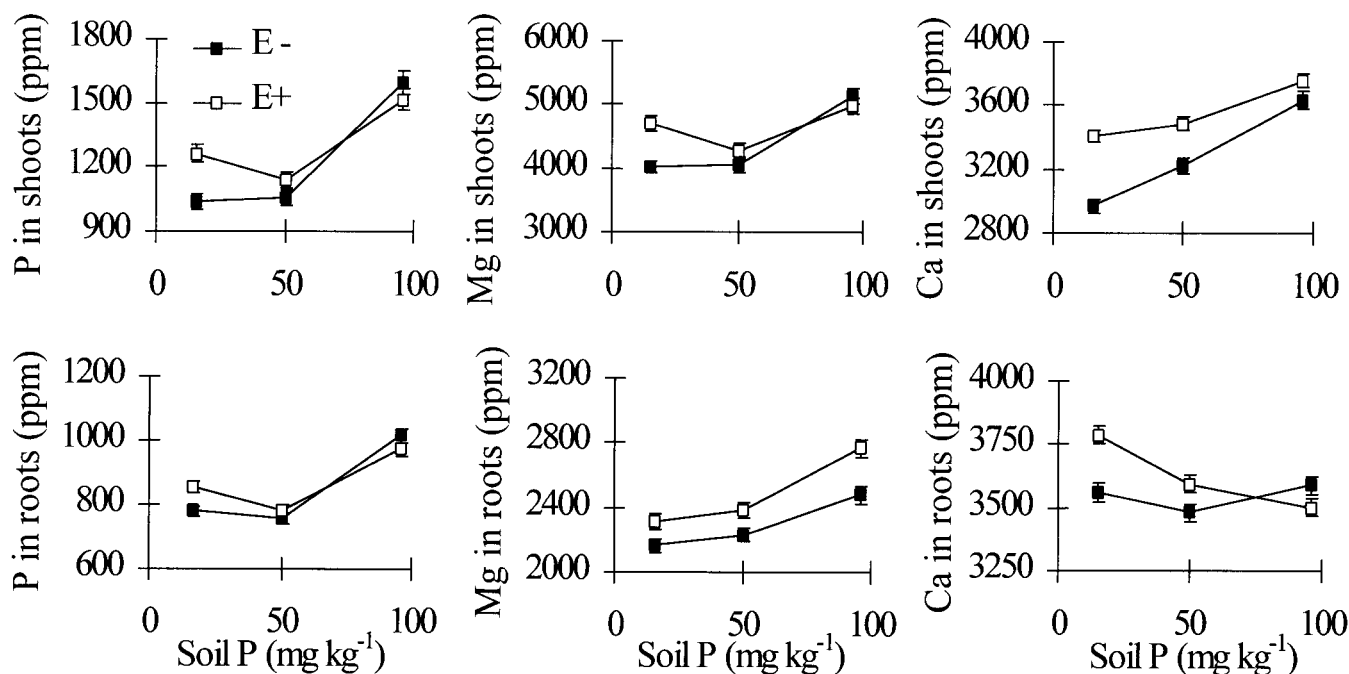


Fig. 3. Concentrations of P, Mg, and Ca in roots and shoots of endophyte-infected (E+) and noninfected (E-) tall fescue (averaged over four genotypes) as a function of soil P availability. Adapted from Malinowski et al. (1998b).

at a location with the highest soil N concentration (about 2.0 mg kg<sup>-1</sup>). These results suggest that endophyte benefits in cool-season grasses depend on specific endophyte-host associations and site nutrient availability conditions.

### Phosphorus

Phosphorus is involved in ergot alkaloid biosynthesis in *Claviceps* spp., a fungus related to clavicipitaceous grass endophytes (Robbers, 1984; Flieger et al., 1991). A high P concentration restricted the activity of the first enzyme in the biosynthesis pathway of ergot alkaloids, dimethylallyl tryptophan synthase (DMATase), in *in vitro* grown *Claviceps* spp. A similar pathway of ergot alkaloid biosynthesis is proposed for grass endophytes within the Clavicipitaceae (Garner et al., 1993; Glenn and Bacon, 1997). Phosphorus nutrition, therefore, has been postulated to affect ergot alkaloid production and growth of *Neotyphodium* spp. infected grasses (Porter, 1994).

Detailed studies on P involvement in growth and ergot alkaloid production in endophyte-infected tall fescue were initiated by Azevedo (1992) and Azevedo et al. (1993). The authors investigated two cloned genotypes of Kentucky-31 tall fescue, DN7 and DN11, grown at contrasting soil P levels determined as deficient (0.02 mg kg<sup>-1</sup> P) to sufficient (2.0 mg kg<sup>-1</sup> P). These tall fescue clones produce low (DN7) and high (DN11) amounts of ergot alkaloids when endophyte-infected, and have been used extensively to study endophyte effects on tall fescue growth and physiology (Belesky et al., 1989a,b; Hill et al., 1990, 1996). Growth and photosynthesis rates of both endophyte-infected tall fescue genotypes were reduced compared to noninfected plants as P availability in the soil increased. Endophyte appeared to be a sink for P (Azevedo, 1992). The production of ergovaline was a function of increased P availability and was not

related to specific endophyte strain-tall fescue genotype association (Azevedo et al., 1993). In a subsequent study (Azevedo and Welty, 1995), endophyte hyphae accumulated inorganic P, similar to that found in mycorrhizal fungi (White and Brown, 1979; Lapeyrie et al., 1984). Such P reserves in endophyte hyphae might benefit grass host under low soil P conditions.

Our research group conducted a series of experiments to clarify the role of endophyte in tall fescue tolerance to P deficiency. We grew four tall fescue cloned genotypes (DN2, DN4, DN7, and DN11) infected with endemic endophytes and their noninfected clones in an acidic, high Al content soil at three P levels of 17, 50, and 96 mg kg<sup>-1</sup> (Malinowski et al., 1998b). The P levels represented low, medium, and high P fertilization rates appropriate for acidic soils (Wright et al., 1987). Endophyte-infected plants had less root and shoot DM compared with noninfected plants as P availability increased (Fig. 2). At low and medium P levels, specific root length of endophyte-infected tall fescue was considerably greater than that of noninfected plants suggesting a finer root system. Concentrations of P, magnesium (Mg), and calcium (Ca) in roots and shoots were greater in response to endophyte infection only when plants were grown at low soil P level (Fig. 3). Concentrations of ergot alkaloids were increased in low ergot alkaloid genotypes DN2 and DN4 as P fertilization increased. In contrast, ergot alkaloid production in high ergot alkaloid genotypes DN7 and DN11 reached a peak at medium soil P level and declined with a further increase in soil P availability. Interestingly, Al concentration in roots was greater than in shoots of endophyte-infected plants compared with noninfected plants, suggesting an Al sequestration mechanism located in/on the roots of endophyte-infected tall fescue. The results show that endophyte infection does not benefit tall fescue growth as P availability in the soil increases, and that manipulat-

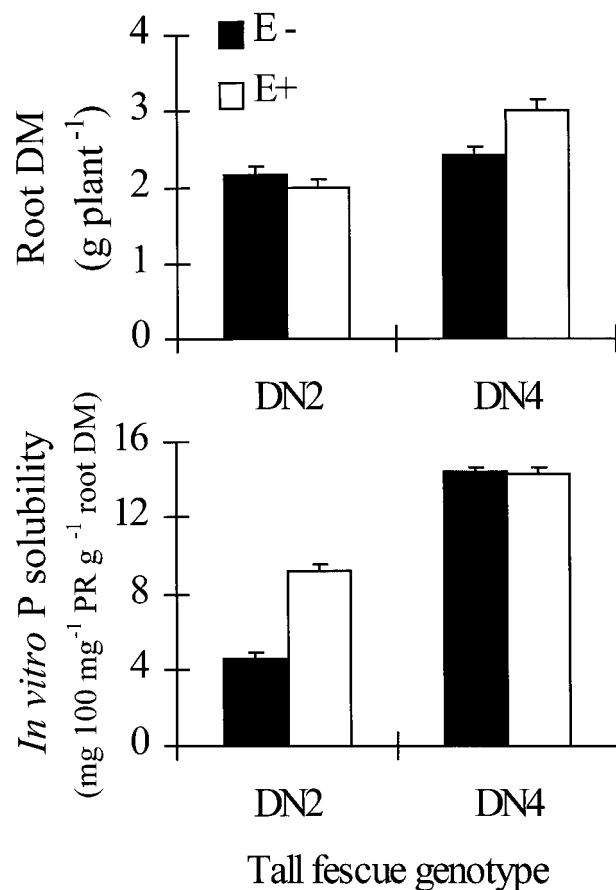


Fig. 4. Root DM of tall fescue genotypes DN2 and DN4 grown in a P-deficient soil supplied with phosphate rock (PR) and in vitro P solubility (from PR) by tall fescue root exudates in response to infection with the leaf fungal endophyte, *N. coenophialum* (E<sup>-</sup>, noninfected; E<sup>+</sup>, infected). Adapted from Malinowski and Belesky (1999a).

ing P nutrition may reduce or increase production of ergot alkaloids, depending on particular endophyte–tall fescue associations.

Negligible responses of root and shoot DM to endophyte infection in tall fescue grown at low soil P level and increased concentrations of P, Mg, and Ca in plant tissues suggested an endophyte-related mechanism of mineral uptake. In a subsequent study (Malinowski and Belesky, 1999a), we investigated growth of two tall fescue genotypes (DN2 and DN4) at low (about 10 mg kg<sup>-1</sup>) soil P level where P was supplied in a readily available form of commercial fertilizer or less available form of phosphate rock. Endophyte-related increase in root DM (10%), relative growth rate (16%), and P uptake rate (24%) were observed in plants grown with phosphate rock but no significant effects of endophyte were observed in plants grown with commercial P fertilizer. Interestingly, endophyte infection increased root growth in tall fescue genotype DN4 but no significant effect was observed in genotype DN2 grown with phosphate rock (Fig. 4). In contrast, the activity of root exudates in dissolving P from phosphate rock was increased by 100% in response to endophyte infection in DN2 but no endophyte effects were observed in DN4 (Fig. 4). We concluded that endophyte might induce at least two mechanisms of mineral uptake in response to P deficiency: alteration in root morphology and activity

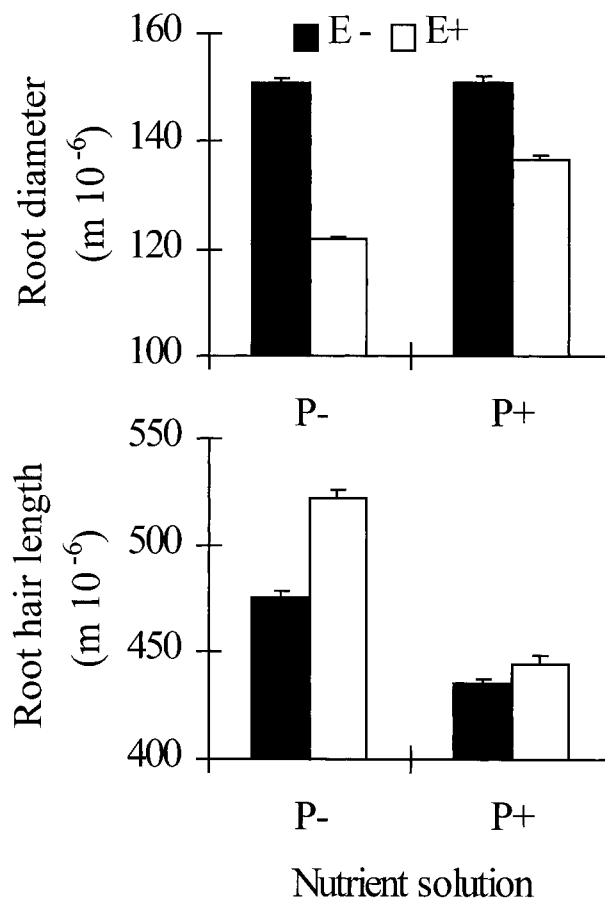


Fig. 5. Root diameter and root hair length of tall fescue (averaged over genotypes DN2 and DN4) grown in P-deficient and P-sufficient nutrient solution as a function of endophyte infection (E<sup>-</sup>, noninfected; E<sup>+</sup>, infected). Adapted from Malinowski et al. (1999b).

of root exudates. Both of those mechanisms might be differently expressed in a particular endophyte–tall fescue association.

We cultivated the same endophyte–tall fescue associations in P-deficient (0.31 mg P L<sup>-1</sup>) and P-sufficient (31 mg P L<sup>-1</sup>) nutrient solutions (Malinowski et al., 1999b). In response to P deficiency, endophyte-infected plants produced roots with smaller diameters and longer root hairs (Fig. 5), which may increase the root surface area that functions in mineral acquisition (Foehse and Jungk, 1983). These endophyte-related adaptations in root morphology of hydroponically grown tall fescue to P deficiency agreed with our previous observations of a modified specific root length in soil-grown tall fescue (Malinowski et al., 1998b). Therefore, we proposed longer root hairs and smaller root diameters of endophyte-infected plants as one mechanism of P deficiency tolerance induced in tall fescue by its endophyte.

We were interested in the nature of exudates released by tall fescue roots in response to endophyte infection under P-deficient conditions (Malinowski and Belesky, 1999a). On the basis of our preliminary observations on genotypes DN2, DN4, DN7, and DN11, we applied a technique to visualize chemical changes in the rhizosphere of tall fescue in response to endophyte infection and P deficiency (Malinowski et al., 1998a). Roots of endophyte-infected plants released exudates (identified

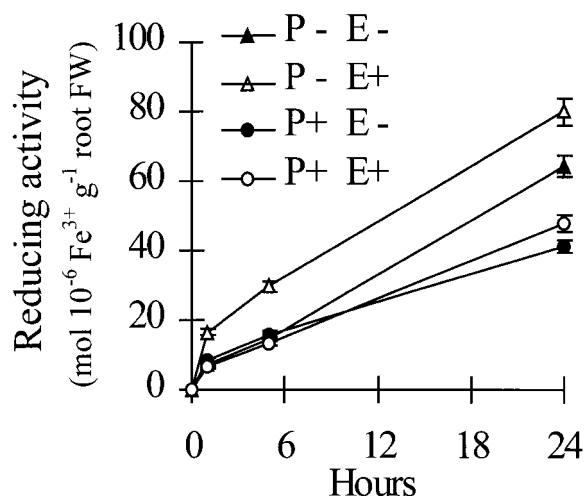


Fig. 6.  $\text{Fe}^{3+}$  reducing activity of exudates from intact roots of tall fescue (averaged over genotypes DN2, DN4, DN7, and DN11) in response to endophyte infection (E-, noninfected; E+, infected) and P availability in the growth medium during a 24-h measurement cycle. Adapted from Malinowski et al. (1998a).

as phenolic-like compounds) with a higher iron ( $\text{Fe}^{3+}$ ) reducing activity than those of noninfected plants (Fig. 6). The  $\text{Fe}^{3+}$  reducing activity was located on the root surface and limited to the zones of active nutrient uptake. Concentrations of phenolic-like compounds in roots and shoots of endophyte-infected tall fescue were greater than those in noninfected plants only under P-deficient conditions (Fig. 7). A release of phenolic-like compounds by tall fescue roots was previously reported by Creek and Wade (1982) in plants with undetermined endophyte status. Phenolics can increase P availability in the soil by binding soluble Al, Fe, and manganese (Mn), which can otherwise render P unavailable (Kafkafi et al., 1988; Appel, 1993). The mechanism responsible for production of phenolic-like compounds by endophyte-infected tall fescue in response to P deficiency is not known. We suggest that endophyte, despite the mutualistic relationship, may induce an internal stress in tall fescue and, under certain stress conditions, trigger a range of defense mechanisms in the host plant similar to those induced by infection with pathogenic fungi. Circumstantial evidence supports our hypothesis. Powell et al. (1994) reported an endophyte-related increase in the concentration of resveratrol, a phenolic compound, in a range of cool season grasses. Resveratrol has antifungal activity and has been correlated with disease resistance in plants (Siemann and Creasy, 1992). Chitinase, a pathogenesis related protein, was found in greater concentrations in roots and shoots of endophyte-infected tall fescue compared with noninfected, genetically identical conspecifics (Roberts et al., 1992). Occurrence of resveratrol and chitinase suggests that endophyte may influence the host to induce a range of defense responses.

### Aluminum

Foy and Murray (1998) showed that tall fescue accumulated considerably less Al in shoot than root tissues, suggesting an efficient mechanism for Al sequestration in or on the roots. The endophyte status in experimental plants was not determined by the authors. Liu et al.

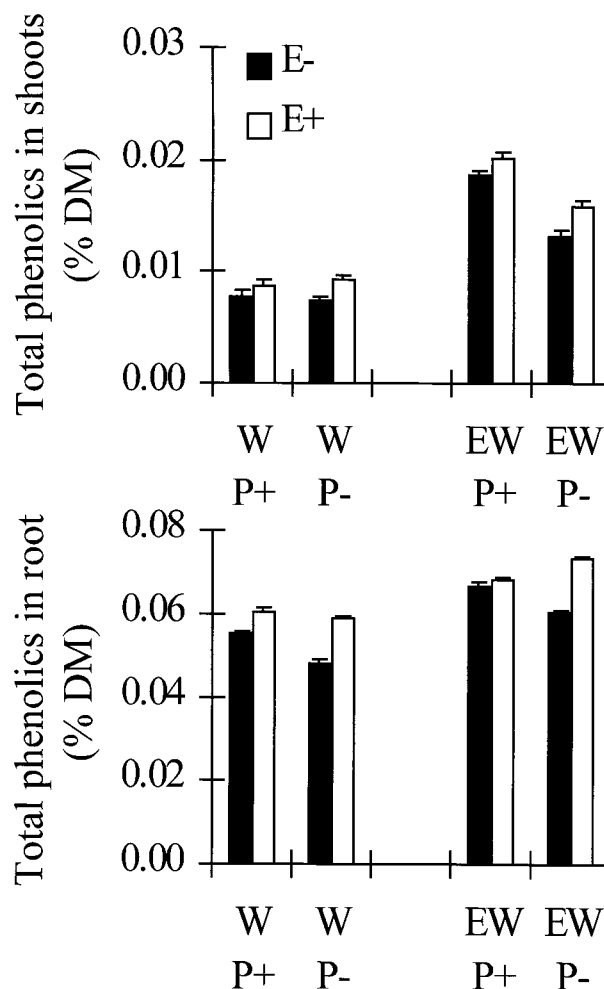


Fig. 7. Concentrations of water soluble (W) and ethanol:water (50:50 v/v) soluble phenolic compounds (EW) in roots and shoots of endophyte-infected (E+) and noninfected (E-) tall fescue (averaged over genotypes DN2, DN4, DN7, and DN11) as a function of P availability in the growth medium. Adapted from Malinowski et al. (1998a).

(1996) observed greater Al tolerance in endophyte-infected fine fescues (*Festuca* spp.) compared with noninfected plants in terms of DM accumulation. On the basis of our previous findings (Malinowski et al., 1998a), we suggested that phenolic-like compounds released in greater amounts by roots of tall fescue in response to endophyte infection and mineral stress might contribute to Al stress tolerance. Phenolics can chelate Al (Marschner, 1986) and may facilitate transport of Al within a plant in nontoxic forms (Taylor, 1988). We evaluated two (DN2 and DN4) of the endophyte-tall fescue associations investigated by Malinowski et al. (1998a) for exudation of phenolic-like compounds by roots under high Al (640  $\mu\text{M}$  Al) conditions (Malinowski and Belesky, 1999b). Endophyte infection did not affect DM production of Al-stressed tall fescue but the relative (to no Al) reduction in root and shoot DM was greater in endophyte-infected than noninfected plants. This suggested a negative effect of endophyte infection in terms of DM production under Al stress. Concentrations of Al in shoots were not affected by endophyte and were about 10 fold less when compared with Al concentrations in roots, suggesting an Al sequestration



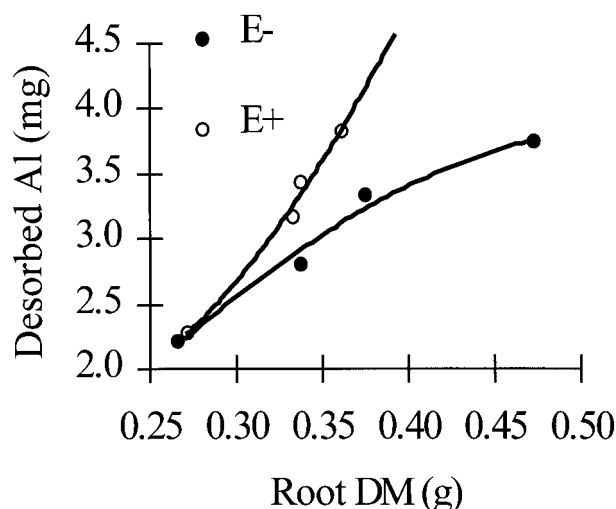


Fig. 8. Relationship between root DM and amount of Al desorbed from root surfaces of tall fescue (averaged over genotypes DN2 and DN4) as a function of endophyte infection (E-, noninfected; E+, infected). Adapted from Malinowski and Belesky (1999b).

mechanism as proposed by Foy and Murray (1998) and Malinowski et al. (1998b). Roots of endophyte-infected plants accumulated 35% more Al than did roots of noninfected plants (excluded Al removed from root surfaces by citric acid). Most of the total Al (94%) could be removed from root surfaces and cortical free spaces by citric acid, which confirms an efficient sequestration mechanism. The concentration of Al desorbed from root surfaces was 46% greater in response to endophyte infection. Calculated on a basis of root DM, more Al was sequestered on root surfaces of endophyte-infected plants compared with noninfected plants (Fig. 8). We did not analyze phenolic-like compound concentrations in this experiment. On a basis of our previous results (Malinowski et al., 1998a), however, we suggest that phenolic-like compounds may contribute to increased Al sequestration on tall fescue roots in response to endophyte infection. Increased production of root exudates in response to mineral stresses requires a high energy cost to the plant (Smucker, 1984). Therefore, a reduction in DM of endophyte-infected plants in response to Al stress may be related to the increase in exudation of phenolic-like compounds to alleviate toxic effects of Al in the rhizosphere. This mechanism should be considered in future research on endophyte-related tolerance to Al stress in tall fescue.

### Other Mineral Elements

Responses of endophyte-infected grasses to mineral stress other than N, P, and Al have received minimal attention. Calcium is another mineral which may be affected by endophyte. Calcium, along with N and P, is involved in ergot alkaloid biosynthesis as a coenzyme for DMATase (Lee et al., 1976; Cress et al., 1981). Preliminary studies showed that Ca fertilization increased ergot alkaloid concentration in tall fescue regardless of the soil pH (Hill, 1995). We observed increased specific absorption rates for Ca, P, Mg, K, and some microelements in one endophyte-infected tall fes-

cue genotype (DN2) but the responses were not consistent in another genotype (DN4) (1998, unpublished data). This confirms a frequently reported specificity of endophyte–grass associations in responses to environmental stresses. Copper (Cu) concentration was lower in herbage of endophyte-infected Kentucky 31 tall fescue grown in greenhouse (Dennis et al., 1998). Allen et al. (1997) observed the same response in field experiments in Virginia, but an opposite one in Mississippi. Steers fed endophyte-infected tall fescue forage with low Cu had reduced Cu concentrations in serum compared with steers fed noninfected forage (Saker et al., 1998). Apparently, uptake of mineral elements by symbiotic plants may be controlled by a range of factors including tall fescue genotype, endophyte strain, and environmental conditions.

An endophyte-enhanced release of phenolic-like compounds with  $\text{Fe}^{3+}$ -reducing activity into the rhizosphere (Malinowski et al., 1998a) may affect Fe uptake by tall fescue. The mechanism of Fe uptake in grasses involves nonproteinogenic amino acid carriers (phytosiderophores) (Tagaki, 1976; Marschner, 1986). Recently, an alternative mechanism has been reported for mycorrhiza-infected sorghum [*Sorghum bicolor* (L.) Moench] (Caris et al., 1998). The authors reported a direct involvement of mycorrhizal fungi in Fe mobilization or uptake and transfer into the host plant. Phytosiderophores exuded by grass roots form highly stable complexes with  $\text{Fe}^{3+}$  (Mino et al., 1983) but not with  $\text{Fe}^{2+}$  (Beneš et al., 1983). The  $\text{Fe}^{3+}$  chelates are transported into root cells where  $\text{Fe}^{3+}$  is reduced to  $\text{Fe}^{2+}$  and used in biochemical processes. Because endophyte-infected tall fescue had greater  $\text{Fe}^{3+}$ -reducing activity on root surfaces (Malinowski et al., 1998a) compared with noninfected plants, Fe uptake may be indirectly affected by endophyte. More detailed research is required to resolve the relationship between rhizosphere chemistry and Fe uptake by endophyte-infected tall fescue.

### Soil Acidity

The major factors limiting plant growth on acid soils are increased concentrations of exchangeable  $\text{H}^+$ , exchangeable  $\text{Al}^{3+}$ , and soluble Mn, decreased concentrations of exchangeable  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{K}^+$ , and reduced solubility of P and molybdenum (Mo) (Marschner, 1991). Low soil pH and high exchangeable Al concentration restrict root growth, N uptake, and N transport within grass plants (Thornton, 1998). Research on endophyte effects on tolerance to acid soils is very limited. Under simulated acid rain, Cheplick (1993) observed a reduced productivity of tall fescue in response to endophyte infection. In contrast, some endophyte-infected tall fescue genotypes (DN2 and DN7) grown in acidic, high exchangeable Al content soil produced greater root systems than did noninfected plants while other genotypes (DN4, DN5, and DN11) did not respond to endophyte infection (Belesky and Fedders, 1995). Malinowski and Belesky (1999a) observed that the pH of a limed, acidic soil increased faster as a result of root activity of endophyte-infected tall fescue compared with noninfected

plants under P deficient conditions. We observed an increase in the pH of a P-deficient ( $0.31\mu\text{g mL}^{-1}$  P) nutrient solution from 6.0 to 7.5 within 3 d, regardless of endophyte status of tall fescue plants (1998, unpublished data). In contrast, changes in the pH of a P-sufficient ( $31\mu\text{g mL}^{-1}$  P) nutrient solution were dependent on specific endophyte–tall fescue associations. This suggests that rhizosphere chemistry may be affected in different ways depending on endophyte and tall fescue genome interactions.

### **Manipulating of the *Neotyphodium*–Grass Association for Beneficial Uses**

The responses of *Neotyphodium* endophyte–grass associations are often inconsistent and are a function of a particular association, as well as extrinsic factors. This creates a high degree of plasticity in populations of endophyte-infected tall fescue, which enables the population to persist under a range of growing conditions and management practices. Variable responses suggest that expression of characteristics may be regulated by interactions or products of multiple genes of the endophyte as well as the grass host genomes. This complicates the approach required to obtain the beneficial aspects of the association. Should the endophyte, the grass host or the association be manipulated? Hill et al. (1991) identified a strong contribution of the plant genome in controlling the expression of ergopeptine alkaloid production by the endophyte. This suggests that ergoalkaloid production patterns of isolated endophyte strains may be unpredictable once they are introduced into tall fescue hosts. Similar results were reported for short- and long-term drought acclimation in tall fescue, indicating that plant chemistry as well as physiology were highly dependent on a particular endophyte–plant genome interaction (Hill et al., 1996). The highly plastic endophyte–host grass association suggests that further research be conducted at the population level rather than with specific associations to understand plant population responses (West, 1994). Before this can be achieved, it is important to identify mechanisms operating in endophyte–grass associations that might be sensitive indicators of change. Investigations should be conducted with highly responsive endophyte–host grass associations to isolate genes involved in the expression of beneficial traits. The long-term impact of genetically modified *Neotyphodium*–tall fescue associations on plant diversity (Clay and Holah, 1999) must be considered and balanced with the relatively short-term economic returns from improved pasture productivity.

### **Symbiosis of Cool-Season Grasses with *Neotyphodium* spp. Fungal Leaf Endophytes and Environmental Stress Tolerance—An Alternative Strategy to VA Mycorrhiza?**

Endophyte-infected grasses are better adapted than noninfected grasses to abiotic stresses, i.e., drought and marginal soil conditions. Results presented in this review show that *Neotyphodium* spp. endophytes induce direct changes affecting water status in shoots and indi-

rect changes in root morphology and function. These adaptations may arise from a chemical signaling system in the symbiotum. Apparently, drought or mineral stress signals registered by roots can be received by endophyte and induce a range of responses in the host plants which may affect the uptake and transport of water and nutrients. Less is known about the chemistry of these signals at this time. Benefits arising from symbioses between leaf fungal endophytes and grasses, in terms of drought and mineral stress tolerance, are similar to well-known benefits of symbioses between mycorrhizal fungi and grasses. Mycorrhizal fungi can alter water relations of the host grass and enhance drought tolerance (for review see Miller, 1987; Smith and Read, 1997) by increased root surface area via mycorrhizal hyphae, and increased water uptake by hyphae (Ruiz-Lozano and Azcón, 1995). Endophyte-infected grasses increase root surface area by reducing root diameter and producing longer root hairs compared to noninfected grasses (Malinowski et al., 1999b). The presence of mycorrhizal hyphae in roots increased leaf gas exchange as the result of improved water transfer (Hardie, 1985; Ruiz-Lozano and Azcón, 1995). Endophyte effects on gas exchange in grasses are opposite to that of mycorrhizal plants (Elbersen and West, 1996). Mycorrhizal grasses can better acquire water compared with non-mycorrhizal grasses as soil water deficit increases; therefore, grass growth is less diminished during brief periods of drought (Ebel et al., 1994). In contrast, endophyte-infected grasses survive drought by reducing transpiration losses compared with noninfected plants. This adaptation may ensure drought survival but not necessarily better growth of endophyte-infected grasses compared to noninfected conspecifics. These two strategies differ from each other; however, they both enhance survival of symbiotic plants. Altered hormonal status in grasses, as an effect of fungal infection, may also affect water status regulation (Dannenberg et al., 1992; Joost et al., 1993). Increased nutrient uptake, particularly that of P, by symbiotic grasses in response to mineral stress occurs because of chemical modification of the rhizosphere directly by the mycorrhizal hyphae (for review see Smith and Gianinazzi-Pearson, 1988) or indirectly by alteration of root chemical activity in endophyte-infected grasses (Malinowski et al., 1998a). Although there are substantial differences between mycorrhizal grasses and endophyte-infected grasses, both types of the symbiosis benefit plants grown in mineral deficient soils and under drought, contributing to their survival and reproductive success compared with nonsymbiotic plants. These two different symbiotic associations of grasses with fungi might be antagonistic in tall fescue (Barker, 1987; Chu-Chou et al., 1992; Guo et al., 1992) suggesting alternative strategies to ensure plant survival under suboptimal environmental conditions.

### **CONCLUSIONS**

Mutualistic symbiosis of grasses with shoot-localized *Neotyphodium* spp. endophytes contributes to adapt-

ability and subsequent widespread persistence of the associations in challenging edaphic and environmental conditions. Infection with *Neotyphodium* spp. endophytes induces changes in host grass physiology, especially in root morphology and root function. These changes may be a result of intricate signaling and bio-feedback systems involving the symbionts. Compounds produced as a function of endophyte infection, such as pyrrolizidine alkaloids in tall fescue, may function in both biotic (anti-herbivory, allelopathy) and abiotic (osmoregulation) stress resistance mechanisms. Endophyte-infection induced root exudates appear to have the same dual stress resistance function in tall fescue. These root exudates may contribute to nematode resistance and apparent allelopathic effects on competitors in mixed swards (biotic stress) and metal ion chelation affecting sequestration and availability (abiotic stress). This facet of symbiont response requires further investigation to realize the full benefit of endophyte–tall fescue associations surrogately transformed to modify ergo-alkaloid production. The nature of communication between endophyte and host grass remains to be determined.

Defining the responses of endophyte–grass associations to various extrinsic factors is fundamental to understanding pasture community structure and ultimately, success of the population. Further careful investigation of surrogately transformed endophyte–grass associations is necessary if the valuable attributes of such associations should be considered for improvement in tolerance of cool-season grasses to environmental stresses. Close linkage between shoot–root function and evolving knowledge of how endophytes mediate the relationship, complicates our extrapolation of individual behavior to community level responses. Modifying endophyte–host relationships for a specific factor may have unknown consequences for association persistence, once multi-faceted extrinsic factors and trophic levels are considered.

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